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[200] HUMAN PATHOLOGY AND IN VITRO STUDIES

The recognition of diseases caused by inhalation of asbestos dust was originally based on histopathologic observations of the lung. In 1906, Montagu-Murray reported to a departmental committee on industrial diseases in England concerning a case of pulmonary fibrosis in an asbestos worker on whom he had done an autopsy (373). This case was not published in the literature and so were many other autopsies performed in the 1880s at a hospital in Turin. This compiler is in the process of obtaining these records to document the fact that the first cases of asbestosis diagnosed by gross and microscopic observations of the lung occurred in Italy in the 1880s, rather than in England in 1906.

The first published case of lung cancer in a patient suffering from pulmonary asbestosis was reported in 1935 by an American pathologist, and additional cases were reported from England and Germany. Thus, prior to the Second World War, there was enough scientific information indicating that occupational exposure to asbestos can cause diseases of the lung, such as pulmonary fibrosis and bronchogenic carcinoma, demonstrable by histopathologic studies.

A 1982 monograph released by the Commercial Union Insurance Companies which is intended to examine the scientific evidence on Asbestos, Smoking and Disease (374) does not discuss the early recognition of asbestos diseases, although the relationship between tobacco and cancer was reviewed. The monograph traces its origin to at least 200 years ago, in an article describing nasal lesions caused by the use of snuff. The relationship between cigarette smoking and lung cancer is attributed to a 1929 publication by Tylecote, and to a 1939 article by Ochsner and DeBakey. It should be recognized that these two articles contained clinical impressions without any case reports. The epidemiologic evidence associating lung cancer and cigarette smoking was not available until the 1950s. A review of the literature conducted by this compiler suggests that recognition of the pathogenetic effects of asbestos dust exposure predated by several decades the epidemiologic studies relating to cigarette smoking.

The techniques used to investigate the effects of asbestos dust on human lungs, blood and tissue cultures are discussed below. Compared to animal experiments [Categories 100s], the human studies are less extensive and efforts to either prove or disprove asbestos/smoking interaction concept are minimal. The compiler offers an explanation as follows: human pathologists

generally do not agree with concepts derived from animal experiments and epidemiologic observations upon which the interaction hypothesis is based.

[210] Histopathologic Features of Pulmonary Asbestosis and Cancer

The first monograph on the subject appeared in 1938, entitled Silicosis and Asbestosis, edited by Lanza (375). The chapters on pathology by Gloyne (376) and on experimental pathology by Gardner (377) summarize the importance of microscopic observations in differentiating between asbestosis and silicosis. Auerbach (378) reviewed the differences in an article appearing in 1937, thirty years prior to his own description of histopathologic changes seen in the lungs of cigarette smokers. This is another example in the expanding list of events indicating that the pulmonary effects of asbestos dust exposure predated those from cigarette smoking.

The specific histopathological changes seen in asbestosis, mesothelioma and bronchogenic carcinoma are discussed under their respective disease categories [400s, 500s and 600s]. It suffices to state here that "despite the elegant techniques in biochemistry, pharmacology and toxicology which have been applied to the investigation of lung cancer, the morphological

parameter is the most accurate measurable index of environmental exposure risk". This opinion was stated by Kotin (379) prior to becoming employed by an asbestos manufacturing company.

Weston et al (380) published in 1972 a collection of cases of inhalational injury to the lung, including asbestos dust exposure. Although there were no diagnostic pathological problem, the medicolegal ramifications which may arise were discussed. The problems in the interpretation of histopathologic changes in the lungs resulting from occupational or nonoccupational exposure to asbestos have been reviewed by Bignon and Bientz (381).

[220] Asbestosis or Asbestos Bodies

Between 1927 and 1929, Cooke and Hill reported the occurrence of "curious bodies" in sections of the lung of a patient who died from exposure to asbestos dust (382-386). They were found in alveoli, bronchioles, fibrous and necrotic areas. The larger bodies measured from 20 to 100 microns or more in length and were of golden brown in color and gave a Prussian blue reaction for iron. They concluded that these bodies contained asbestos particles and originated as tissue reaction, thus the term "asbestosis bodies" to signify that they were pathognomonic of asbestos-induced disease process.

In the 1930s, Gloyne supported the concept of asbestosis bodies as pathognomonic of asbestos disease. He cited unpublished guinea pig experiments conducted prior to 1928 by Mavrogordato, and listed eleven morphologic features of asbestosis bodies (387-389). Since no asbestosis bodies have been found in crude asbestos, Gloyne was certain that they are produced in living tissues from inhaled asbestos fibers.

In 1951, Gloyne (390) reported the incidence of primary neoplasm of the lung in a group of 102 cases of asbestosis, 41 males and 61 females. Postmortem examination revealed an incidence of malignancy in 14.1 percent, consisting of 19.6 percent for males and 9.7 percent for females. He also noted that in long standing cases of asbestosis, both the fibers and bodies were smaller and less often seen than in lungs of workers more recently exposed to asbestos, suggesting that in course of time, the fibers and bodies are slowly dissolved. It should be noted that the majority of deaths took place in the age group of 45 to 64 years in the prime of life when experience in these skilled occupations counted most. These observations are contrasted below with more recent observations of British asbestos workers.

The first reported detection of the curious bodies among American asbestos workers was in 1937 by Lynch. He proposed

the term "asbestos bodies" instead of asbestosis bodies, and "silica bodies" to refer to those associated with silicosis. He also proposed that other fiber or crystalline dusts not yet particularly studied may produce similar bodies and cautioned that confusion of some consequence may result from failure to realize that asbestos bodies may be simulated by other deposits in the lung. German pathologists (392, 393) agreed with Gloyne which in turn prompted a new terminology described in the next category.

Asbestos/Smoking Interaction. From 1927 to 1931, Tylecote published on asbestosis bodies and lung cancer (394, 395). He is also cited (by the Commercial Union Insurance Monograph) as the first pathologist who linked cigarette smoking as a cause of lung cancer. Tylecote's statements were as follows: "I have no statistics with regard to tobacco, but I think that in almost every case I have seen and known of, the patient has been a regular smoker generally of cigarettes. To this there have been the following exceptions: (a) two ladies who succumbed to the disease unusually rapidly. In each case the lady lived 'hard by' a railroad station where trains frequently stopped, and where smoke from the engines must have at almost all times pervaded both house and garden. (b) A group of acute adolescent cases, all males in which I regard the

condition as being usually mediastinal Hodgkin's disease" (395).

The above quotation by Tylecote is regarded as documentation for the claim that lung cancer associated with cigarette smoking preceded the recognition of the disease among asbestos workers. Tylecote was probably the first pathologist who could have detected the phenomenon of asbestos/smoking interaction, if it existed at all, and the same can be stated for Auerbach who was also interested in the pulmonary effects of asbestos and cigarette smoke. Simons and Ah (396) are other pathologists who wrote on etiological concepts of lung cancer and although they mentioned mined dust particulates and cigarette smoke in their list of 14 causes, they did not specify any form of interaction.

[230] Ferruginous Bodies and Asbestos Fibers

Before discussing the changing concepts on the significance of asbestosis or asbestos bodies, it might be helpful to recall a 1935 publication (397) from two Boston investigators (one a petrographer for the Liberty Mutual Insurance Co.). After the appearance of the results of guinea pig studies of Gardner and Cummings [Category 111] and human histopathologic observations showing the persistence of asbestos fibers in the lung [Category 220], the petrographers conducted a

photomicrographic analysis of rafter dust from six asbestos processing plants that included molding and weaving of asbestos fibers. They concluded that in the respirable sizes (less than 10 microns in maximal diameter for particles, or less than 5 microns in thickness for fibers as long as 200 microns), asbestos is a relatively "unimportant constituent". Instead, the predominating constituents of respirable dusts were talc, serpentine, carbonate and magnetite. These results were not challenged for three decades until additional research studies in human lung revealed the deposition of significant amounts of respirable asbestos fibers in the lungs of asbestos exposed workers.

[231] Tissue Analytical Methods

There were two significant events that resulted in the improvement in analytical methods for asbestos analysis in human tissues. First, Gross et al in 1967 questioned the specificity of asbestos bodies and suggested that the iron containing structures be called "ferruginous bodies" since they are formed in response to the inhalation of filamentous dust of unknown composition (398-401). Secondly, research was being directed towards detection of asbestos fibers which was prompted by the results of Knox and Beattie (402, 403). They measured the

mineral content of the lungs from 27 workers in the asbestos-textile industry. Like earlier British investigators, they reported that the mineral material found in the lungs increased in amount as the exposure time lengthened. As the survival time increased, the mineral content of the lung tended to decline. They also questioned the significance of the mineral content which was unrelated to the severity of asbestotic lesions in the lungs. On the other hand, since severity was related to the sum of exposure time and survival time, the results of animal studies were questioned by Knox and Beattie.

The above results reported in 1954 were derived from light microscopic measurements of particle size distributions contained in the incombustible and acid insoluble residues of lung parenchyma. In subsequent years, new in situ particle identification methods were introduced that included electron microscopy, both transmission and scanning, back scattered electron imaging, and x-ray mapping. Tissue removal techniques included high-temperature ashing, microincineration, plasma ashing and etching, wet chemical digestion and enzyme digestion. The selection of analytical techniques for the study of dust extracted from the lung and other tissues is dictated by the amount of tissue sample available. Techniques such as quantitative x-ray spectrometry, x-ray fluorescence

spectrometry, and plasma spectrometry are of value in determining elemental concentrations. For the positive identification of asbestos, x-ray diffractometry and selected area electron diffraction are the methods of choice (404-408). The interpretations of results generally disagree with those of Knox and Beattie and there are as many opinions as there are investigators prefering a combination of techniques.

[232] Pulmonary Asbestos Content Among Occupationally Exposed Groups.

The results of pulmonary tissue analysis are briefly listed below. The entries are arranged according to the geographical location of the autopsied subjects or lobectomized patients who were the sources of analyzed lung samples. The nature of occupational exposure and pulmonary pathological lesions showing the presence of asbestos are also indicated.

COUNTRY AND INVESTIGATORS	OCCUPATIONAL EXPOSURE	PATHOLOGICAL LESIONS
United States; see also [232] and [300]		
(404) Mace et al 1980	asbestos workers	asbestos bodies
(406) Gaensler & Addington 1969	shipyard pipe coverer asbestos sheet worker	lung carcinoma
(408) Vallyathan et al 1980	machinist; isolation worker	asbestos bodies

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Australia		
(409) Milne 1971	shipyard workers	asbestos bodies lung carcinoma
France		
(410) Bignon 1970 (411) Jaurand et al 1976 (412) Thomassin et al 1980 (413) Sebastien et al 1980	asbestos workers	pleural fibers parenchymal fibers
(414) Le Bouffant 1974 (415) Le Bouffant et al 1976	asbestos workers	pleural mesothelioma
(416) Fondimare & Desbordes 1974	asbestos workers	asbestos bodies pulmonary fibrosis mesothelioma
(417) Lavoinne et al 1976	asbestos workers	nickel content
(418) Berry et al 1976	miners	ferruginous bodies
Germany		
(419) Friedrichs & Otto 1981	asbestos workers	asbestos fibers mesothelioma asbestosis & cancer
Great Britain		
(402) Knox & Beattie 1954 (403) Knox & Beattie 1954	textile workers	mineral content
(420) Blount et al 1966	asbestos workers	protein content of asbestos bodies
(421) Davis 1964	asbestos workers with lobectomy	asbestos bodies corelation with guinea pig
(422) Ashcroft 1968 (423) Ashcroft & Heppleston 1973 (424) Ashcroft & Heppleston 1973	asbestos workers	asbestos fibers asbestosis

(425)	Henderson et al 1969	asbestos workers	asbestos fibers boiler makers mesothelioma
(426)	Pooley et al 1970	asbestos workers	asbestos fibers
(427)	Pooley 1979		
(428)	Pooley & Clark 1980		
(429)	Pooley & Clark 1979		
(430)	Gaudichet et al 1980		
(431)	Wagner 1973	asbestos workers	asbestos fibers
(432)	Oldham 1973		asbestosis
(433)	Timbrell 1980		
(434)	Lawther 1971	asbestos workers	asbestos bodies
(435)	Acheson & Gardner 1980	asbestos workers	asbestos fibers mesothelioma
(436)	Morgan & Holmes 1980	asbestos workers	mesothelioma
(437)	Morgan & Holmes 1982	gas mask workers	coated & uncoated fibers
India			
(438)	Narang 1980	asbestos workers	asbestos bodies
Italy			
(439)	Governa & Vadala 1972	asbestos workers	asbestos bodies
(440)	Governa & Rosanda 1972		asbestosis
Netherlands			
(441)	Planteydt 1973	asbestos workers	asbestos bodies
(442)	Stumphius & Meyer 1968	shipyard workers	asbestps bodies mesothelioma
Norway			
(443)	Glyseth et al 1979	insulation workers	asbestos fibers
(444)	Ophus et al 1980	shipyard workers	mesothelioma
(445)	Gylseth et al 1981		pleural plaques
(446)	Glyseth & Baunan 1981		
South Africa			
(447)	Goldstein & Rendall 1970	asbestos miners	ferruginous bodies

(448) Thomas & Sluis-Cremer 1977	asbestos miners	asbestos fibers pleural plaques
Switzerland		
(449) Bossard et al 1980	asbestos workers	asbestos fibers

[233] Pulmonary Asbestos Content in American Population Groups

The analysis for asbestos of lung tissues obtained from the nonasbestos workers undergoing autopsy has revealed varying levels that are dependent on the technique used. Although the results reported in the 1960s showed detectable asbestos fibers in 41 percent of autopsied cases, subsequent reports approached incidence rate of 100 percent. There is agreement that the presence of asbestos fibers reflects environmental contamination from sources discussed elsewhere [Category 800].

That asbestos fibers from nonoccupational sources can be deposited in the lung raises a serious question as to the source of carcinogenicity developing in asbestos workers. Are the carcinogens entirely derived from the work place or predominantly from environmental sources? There are known dimensional differences between asbestos fibers in the work place compared to asbestos contaminants in the atmosphere. Furthermore, asbestos fibers are likely to adsorb outdoor pollutants. If the

single target theory of carcinogenesis is true, then it is difficult to prove that the sole source of carcinogens is the work place. On the other hand, if the concept of cocarcinogenesis or synergism is true, what about the interaction between asbestos fibers occupationally inhaled and asbestos fibers from enviromental sources? In other words, the interaction concept of asbestos in work place/asbestos in environment can possibly replace the asbestos/smoking hypothesis. The studies on asbestos content of lung samples derived from autopsies of nonasbestos workers are as follows:

STATE AND INVESTIGATORS	PULMONARY INCIDENCE	PULMONARY LESIONS
Pennsylvania		
(450) Cauna et al 1965	asbestos bodies 41%	
(451) Gross et al 1969		
(452) Davis & Gross 1973		
New York, New Jersey and Minnesota		
(453) Suzuki & Chung 1969		
(454) Langer et al 1970		
(455) Selikoff & Hammond 1970	asbestos bodies 100%	
(456) Langer et al 1973		
(457) Langer & Pooley 1973		
(458) Langer et al 1974		
(459) Auerbach et al 1977		
(460) Selikoff & Lee 1978		
(461) Ehrenreich & Selikoff 1981		
Illinois and California		
(462) Churg & Warnock 1977	asbestos bodies 96%	carcinoma
(463) Churg & Warnock 1977		mesothelioma
(464) Churg et al 1977		

(465) Churg & Warnock 1978
(466) Churg & Warnock 1979
(467) CHurg & Warnock 1979
(468) Churg & Warnock 1979
(469) Churg & Warnock 1979
(470) Warnock & Churg 1980
(471) Churg & Warnock 1980
(472) Churg & Warnock 1981
(473) Churg 1982

Florida
(474) Thomson et al 1966 asbestos bodies 20-30%

Michigan
(475) Dicke & Naylor 1969 asbestos bodies 22%

California
(476) Tabershaw 1968 asbestos bodies 25-50%
(479) Felton 1980

Maryland
(477) Bhagavan & Koss 1976 asbestos bodies 91%

Tennessee
(478) Breedin & Buss 1976 asbestos bodies 90% carcinoma

The above list includes nine publications by Selikoff in collaboration with investigators at the Mt. Sinai Hospital and other institutions. Their concept on the formation of asbestos bodies (460) is different from those of other groups (479, 480). They agree with other groups that asbestos fibers can be derived from environmental sources. In their discussion of forensic significance of asbestos fibers (461) the asbestos/smoking synergism is emphasized without commenting on possible interaction between workplace and environmentally derived

asbestos fibers. The substitute concept of asbestos/asbestos synergism has not been considered in any of the above publications.

[234] Pulmonary Asbestos Content In Foreign Population Groups.

The foreign studies show essentially the same results as the American ones: asbestos fibers and asbestos or ferruginous bodies are present in significant quantities in the lungs of the general population. The lung samples were obtained from consecutive series of hospital autopsies. The reports from thirteen countries are as follows:

COUNTRY AND INVESTIGATORS	PULMONARY INCIDENCE	PULMONARY LESIONS
Australia		
(481) Xipell & Bhathal 1969	asbestos bodies 43.5%	
(482) McCullagh 1978	asbestos bodies	
Canada		
(483) Dionne et al 1976		leiomyosarcoma
(484) Anjilvel & Thurlbeck 1966	asbestos bodies 48%	
(485) Shugar 1979		
Denmark		
(486) Francis et al 1977	asbestos bodies 7%	pleural plaques
France		
(487) Sebastien et al 1977	asbestos bodies 100%	pulmonary fibrosis

Finland		
(488) Meurman et al 1970	asbestos bodies 64%	carcinoma
Germany		
(489) Friedrichs & Otto 1981	asbestos fibers all	
Great Britain		
(490) Lewinsohn 1968	asbestos bodies	
(491) Roberts 1967	asbestos bodies 23%	pleural plaques
(492) Um 1971	asbestos bodies 20%	
(493) Oldham 1973		
(494) Doniach et al 1975	asbestos bodies: men 42% women 30%	
(495) McDonald 1980		
(496) Stovin & Patridge 1982	asbestos fibers all	
Israel		
(497) Polliack 1968	asbestos bodies 26%	
Italy		
(498) Peacock 1968	asbestos bodies 2%	carcinoma
(499) Peacock et al 1969		
(500) Peacock et al 1969		
(501) Ghezzi et al 1967	asbestos bodies 51%	
(502) Bianchi et al 1981	asbestos bodies 94%	pleural plaques
(503) Betta 1982	asbestos bodies: men 58% women 36%	
South Africa		
(504) Thomson et al 1963	asbestos bodies 26%	
Sweden		
(505) Hägerstrand & Seifert 1973	asbestos bodies 47%	pleural plaques
Switzerland		
(506) Stolkin et al 1981		

Yugoslavia
(507) Plamenac et al 1971 asbestos bodies 38%

(508) Dimov et al 1975 asbestos bodies 19%

Asbestos/Smoking Interaction. Although the American publications did not report smoking habits of necropsied subjects [Category 333], the foreign studies include three with such description. The investigation in France included six autopsied cases who were not asbestos workers; one of the two smokers had higher and the other had lower values for pulmonary asbestos content, compared to the three nonsmokers (487). One Italian study (499) did not include a statistical analysis of correlation between reported smoking habit and asbestos bodies because asbestos bodies were rarely detected, a conclusion that is contrary to others conducted in the same country. A Finnish study (488) compared smoking habit and asbestos exposure of lung cancer and control groups. Most of the cases with lung cancer (35 out of 50) belonged to the group of combined tobacco smoking and asbestos exposure, whereas only 20 of the 50 control cases belonged to this group. A statistical calculation of the effect of adding the asbestos body factor to the smoking factor has been made with the collected data. The authors concluded that "it appeared that the combination does not raise the incidence

of lung cancer significantly". This conclusion does not support the asbestos/smoking hypothesis. However the authors explained a lack of additive effect by the following: "The tobacco factor is obviously so strong that the asbestos body factor does not exert any further effect" (488).

[235] Extrapulmonary Tissue Asbestos Content

Analyses of tissues (509) show that ferruginous bodies and asbestos fibers are present in several organs including the larynx (510), gastrointestinal tract and mesothelial tumors (511-515), and kidneys (516). The asbestos fibers could be found not only in asbestos workers but also in the general population. There are no observations relating to the asbestos/smoking interaction concept.

[240] Antemortem Pulmonary Cytologic Observation

In contrast to the preceding sections that relate to postmortem investigations, this section is devoted to the application of the same techniques on samples collected prior to death. Historically, it should be recalled that in the 1930s, asbestosis bodies were reported in sputum of workers by the same investigators who first recognized the disease of asbestos workers in the United States (517), England (518, 519) and South

Africa (520). In the past fifty years, the techniques for cytologic examination have improved and have been applied not only to sputum but extended also to include bronchial lavage and pulmonary biopsy.

[241] Sputum Cytology

The Tyler Asbestos Workers Program, designed to provide a cohort of 890 former asbestos workers that was in operation between 1954 and 1972, is the only American source of data relating to sputum cytopathology. When the Program was initiated in July 1974, approximately 90 percent of the cohort were cigarette smokers which is far in excess of the general population. From 1976 to 1982, ten publications appeared that contain statements supporting the asbestos/smoking synergism (521-530). A close examination of the articles by Greenberg et al reveals the following inconsistencies: First, there was no significant association between the occurrence of ferruginous bodies in the sputum and smoking history. If the interaction hypothesis that smoking inhibits bronchociliary clearance is true [Category 199], then there would be a direct correlation between smoking history and ferruginous bodies in the sputum, since the occurrence of ferruginous bodies was found "to increase as a logarithmic function of the length of occupational

exposure to asbestos in workdays" (524). Second, a lack of association between smoking and ferruginous bodies in the sputum was reversed two years later (526) without any explanation. Third, in another subsequent publication (528), the confusion is compounded by the following statement: "As with specimen quality, smoking was found to influence the detection of ferruginous bodies in the two specimen types. The aerosol-induced specimens from smokers showed a slightly, but not statistically significant, increased yield of ferruginous bodies (34% as compared to 30%, p=0.15). For nonsmokers however, the increased detection of ferruginous bodies in the aerosol induced specimen was found to be significant (29% for aerosol induced as compared to 18% for spontaneous, p=0.05) ... . The smokers in the cohort more frequently produced satisfactory sputum specimens without the aid of the aerosol induction than did the nonsmokers".

It is the opinion of this compiler that the above inconsistent remarks as well as other publications on sputum cytology of smokers versus nonsmokers do not support the interaction hypothesis relating to mucociliary clearance. The conclusions of Greenberg et al relating to asbestos exposure and sputum cytology have not been confirmed by foreign scientists listed below:

COUNTRY AND INVESTIGATORS	OCCUPATIONAL EXPOSURE	SPUTUM CYTOPATHOLOGY
United States		
(521) Greenberg et al 1976 (522) Zeluff et al 1976 (523) Greenberg et al 1976 (524) Farley et al 1977 (525) McLarty et al 1980 (526) Mclarty et al 1980 (527) Roggli et al 1980 (528) McLarty et al 1980 (529) McLarty et al 1981 (530) Greenberg et al 1982	asbestos processing	squamous metaplasia, squamous carcinoma, ferruginous bodies
Egypt		
(531) Ottia et al 1975	asbestos cement pipe workers	cytopathology supported by radiology
Finland		
(532) Huuskonen et al 1978	asbestos processing	squamous metaplasia carcinoma
Netherland		
(533) Planteydt et al 1964	shipyard workers	asbestos bodies
Yugoslavia		
(534) Plamenac et al 1978	asbestos mining	leukocytes, hemosideric macrophages, squamous metaplasia, no ferruginous bodies

Huuskonen et al (532) found ferruginous bodies in sputum colllected from 43 percent of the patients. In addition, they commented on cytopathology: "It is surprising that the smoking

habits in this material appeared to have no effect on the prevalence of benign cellular atypia, possibly either because of the small size of the material or the fact that the heavy effect of asbestos exposure overshadowed the influence of smoking". A similar conclusion was arrived at by the Yugoslavian scientists (534). It is interresting to note that these European studies have not been cited in publications summarizing the results of the Tyler Asbestos Workers Program.

[242] Bronchoalveolar Cytology

Some investigators have relied on bronchial lavage fluid rather than sputum analysis for detection of ferruginous bodies. Bignon et al (535-537) found that results of bronchoalveolar cytology correlate with asbestos exposure better than sputum examination, a conclusion confirmed by investigators from Canada (538), Great Britain (539) and the United States (540, 541). The review article on bronchoalveolar lavage by Gee and Flick (541) summarizes a reasonable correlation between the cellular features of bronchoalveolar lavage and those from lung biopsies in patient with asbestos-induced fibrosis.

[243] Pulmonary Biopsy

Lung biopsy is used to diagnose asbestosis, either by open

surgery (542) or by needle puncture (543-545). The technique has also been used to investigate cellular reactions to inhalation of asbestos. Morgenroth (546) reconstructed the cellular events based on electron microscopy. The smaller asbestos particles are phagocytized by cytoplasm. Larger particles are found either uncoated or coated in the alveoli and in the connective tissue septa. Groups of alveolar macrophages gather around the particles and engulf them. In addition, the asbestos material is dissolved in lacunae appearing in the cellular membrane of macrophages. These ultramicroscopic changes are different from those of cigarette smoke reported by other investigators.

[250] Particulate Deposition in Excised Human Lungs

Since there are discrepancies in results of deposition of particulates in animal and patient studies, physical and mathematical models have been proposed (547-550). Mitchell constructed an artificial chest wall which was large enough to hold an entire excised human lung. He observed extremely good agreement in deposition of aerosol particles with theoretical models (547). So far, the excised human lung or any of the theoretical models have not been applied to the study of

interaction phenomenon.

260] In Vitro Hemolysis

Although the hemolytic effect of asbestos was known since 1914 (551), its significance was not elucidated until fifty years later. Presently, tests of in vitro hemolysis are used to compare cytotoxicity of different forms of asbestos. In conjunction with tests of animal erythrocytes [Category 181], those performed on human cells have been used to support the theories for cytotoxicity. The hemolytic mechanisms derived from in vitro testing of human erythrocytes are as follows:

COUNTRY AND INVESTIGATORS	MECHANISMS OF HEMOLYSIS
United States	
(552) Light & Wei 1977	surface charge
(553) Light & Wei 1977	
(554) Light & Wei 1980	
(555) Schnitzer & Busescu 1970	surface charge and polymer structure
Belgium	
(556) Depasse 1982	sialic acid in membrane lipid
Egypt	
(557) El-Shobaki et al 1973	resynthesis of hemoglobin
France	
(558) Jaurand & Bignon 1979	increased membrane permeability
(559) Jaurand et al 1979	
(560) Jaurand et al 1980	
(561) Jaurand et al 1980	
Great Britain	
(562) Sykes et al 1980	influenced by extracellular osmolarity

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Romania
(563) Gabor & Anca 1975 lipid peroxidation of fatty acids
(564) Gabor et al 1975 surface charge

South Africa
(565) Harington et al 1971 hemolysis enhanced by serum
(566) Harington et al 1971 EDTA interaction

[270] Human Tissue Cultures

Compared to animal organ culture studies [Category 184], those involving human cells are less in number. The results are generally similar in that asbestos particles are cytotoxic, cause macrophageal phagocytosis and fibroblastic proliferation. The importance of trace metals has been examined by leaching and the results indicate that trace metals are not involved in the cytotoxic response. The human tissue culture experiments reported by American, Canadian and European scientists are as follows:

INVESTIGATORS	CELL OR ORGANS	ASBESTOS CYTOTOXICITY
(567) Mace et al 1980 (568) McLemore et al 1980 (569) McLemore et al 1981	alveolar macrophages	phagocytosis asbestos fibers less harmful than naked fibers
(570) Bitterman et al 1981	pulmonary fibroblasts	replication factor
(571) Hart et al 1979 (572) Hart et al 1980 (573) Daniel et al 1980 (574) Hart et al 1980 (575) Tejwani et al 1980	foreskin fibroblasts	ultramicroscopic cytotoxicity benzopyrene interaction

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(576) Lemaire et al 1982	pulmonary fibroblasts	inhibition and proliferation DNA synthesis
(577) Harris et al 1979	bronchus	benzanthracene interaction
(578) Hirsch et al 1982	pleural cells	proliferation
(579) Rajan & Evans 1973	embryonic lung	proliferation
(580) Reiss et al 1980	embryonic intestine	cytotoxicity leaching no influence

Asbestos/Polycyclic Aromatic Amines Interaction. Hart et al (671-575) tested in the human foreskin fibroblast culture the following amines: benzopyrene, dimethylbenzanthrane, nitrosodimethylamine and pyrene. By measuring the concentration ratio of cyclic GMP to cyclic AMP, they concluded that each polycyclic aromatic amine acts as a cocarcinogen to asbestos. The conclusion is based on separate treatment of the cell culture with one substance, rather that the use of a combination. They offer their results in support of the synergism hypothesis between asbestos and cigarette smoking. Harris et al (577) compared asbestos alone and asbestos with a coating of dimethylbenzanthracene. The cultured human bronchial cells were penetrated by both coated and uncoated fibers. There was also hyperplastic epithelial response with cellular atypia but

their publications did not contain any statement that the coated fibers caused more intense changes than uncoated asbestos.

[280] Immunologic Reactions to Asbestos

Contrary to the opinion of asbestos monographers and bibliographers, the first investigation on the effect of asbestos on immunologic events was conducted in 1913 by Coplans (581). The adsorptive properties of asbestos was demonstrated by incubating the substance in blood serum and detected a loss of antityphoid agglutinins and hemolytic complement. The advances in immunology and immunopathology during the past seventy years has led to the proposal that asbestosis and concurrent carcinogenicity are autoimmune diseases. The recent reviews on the immunologic responses as they relate to pulmonary fibrosis (582) and to bronchogenic carcinoma (583) generally highlight the authors' experimental results. Burrell (584) has recently written a commentary on the subject cautioning the enthusiasts. The subcategories on immunologic techniques include in vitro adsorption of plasma constituents, skin testing, lymphocytic reactions, and immunoglobulins.

[281] In Vitro Adsorption of Plasma and Serum Proteins

The list of constituents in the human plasma and serum that is adsorbed by asbestos particles includes the following:

immunoglobulins and globulins (585, 586); coagulation factors XII and VII (587, 588); and complement factors (589, 590). In an in vitro system, the serum reacts with the asbestos particles leading to dissolution of silica (591, 592). It is the opinion of the Indian scientists (591, 592) that the process of dissolution is the basic mechanism for pulmonary fibrosis.

[282] Delayed Hypersensitivity Skin Tests

In four published studies, most if not all of the following skin tests were used: recall test antigens with streptokinase-streptodormase, tuberculin purified protein derivative and Candida albicans extract; and new sensitization testing to dinitrochlorobenzene (583, 593-595). The results show a depression in dermal hypersensitivity and acute dermal responses in patients with pulmonary asbestosis. The depression of dermal hypersensitivity is in agreement with the results of cell-mediated immunity described in the next subcategory.

[283] Cell-Mediated Immunologic Tests

In patients with pulmonary asbestosis, the results of in vitro testing show depression of cell-mediated immunity. The evidence is based not only on lymphocytic profile but also on lymphocytic response to phytohemagglutinin. It should be noted

that results of in vitro tests conform with those of in vivo skin testing. The investigators in the United States, Great Britain, Poland and South Africa are as follows:

COUNTRY AND INVESTIGATORS	CELL-MEDIATED TEST	ASBESTOS EFFECTS IN PATIENTS
United States		
(596) Barbers et al 1981	phytohemagglutinin	in vitro depression
(594) Gaumer et al 1981	skin hypersensitivity	depression in asbesstosis
Great Britain		
(582) Turner-Warwick 1973	lymphocyte responses	depression in
(595) Pierce & Turner-Warwick 1980	skin hypersensitivity phytohemagglutinin	asbestosis
(597) Haslam et al 1978		
(597) Campbell et al '80	phytohemagglutinin	depression in
(598) Kagamimori et al 1980	lymphocytes	asbestosis
(600) Wagner et al 1979		
(601) Wagner 1980		
Poland		
(593) Lange et al 1978	skin hypersensitivity lymphocyte profile	depression in asbestosis
South Africa		
(583) Kagan et al 1978	skin hypersensitivity phytohemagglutinin	depression in asbestosis

Asbestos/Smoking Interaction. Wagner et al (600, 601) investigated the formation of lymphocyte E binding rosettes in

British dockyard workers exposed to asbestos dust. They concluded that there was increased in vitro rosette formation in subjects with radiological evidence of fibrosis who also smoked. They also reported changes in leukocyte and lymphocyte counts that disagree with the observations of cigarette smokers not exposed to asbestos. The publication has several inconsistencies with reported information characterizing smokers. Although Wagner's publication appeared in 1979, there has been no confirmation from other investigators. The significance of rosette formation by lymphocytes has been of interest to some American immunologists but so far, they have not applied the technique to asbestos/smoking interaction.

[284] Humoral Immune Tests

Most studies relating to measurements of immunoglobulins and antibodies show an increase in workers exposed to asbestos compared to controls. There is therefore a paradox in the immunologic responses - an increase in humoral immune indicators but a decrease in cell-mediated immunity [Category 283]. The explanation proposed in 1978 by Kagan et al (583) has not been accepted by others. The studies showing increased humoral mechanisms, including the only negative result from Tyler Asbestos Workers Project (602) are as follows:

COUNTRY AND INVESTIGATORS	HUMORAL IMMUNE TESTS	WORKERS OR PATIENTS
United States		
(602) Nash et al 1981	immunoglobulins	no effect
Egypt		
(603) El-Sewefy et al 1971	serum proteins	reduced in
(604) El-Sewefy et al 1974	bone marrow cells	cement asbestos pipe workers
Finland		
(605) Huuskonen et al 1978	autoantibodies	increased in
(606) Huuskonen et al 1979	immunoglobulins HLA antigens	asbestos workers
Great Britain		
(582) Turner-Warwick 1973	autoantibodies	increased in
(607) Turner-Warwick 1979		asbestos workers
(608) Gregor et al 1979	HLA antigens	no change
(609) Stansfield & Edge 1974	antinuclear antibodies	present in shipyard asbestos workers
Poland		
(610) Lange et al 1974	antinuclear anti-	present in
(611) Matej et al 1977	bodies	asbestosis
(593) Lange et al 1978	IgA, IgG, IgM	higher levels of
(612) Matej et al 1978	HLA antigens	Ig's
(613) Lange 1980		
(614) Lange 1980		
South Africa		
(615) Kagan et al 1977	salivary IgA; se-	increased in
(583) Kagan et al 1978	rum IgA, IgM, IgE	asbestosis

[285] Miscellaneous Tests Including Lymphocytic Hydroxylase Activity.

There are immunologic observations other than cell-mediated or humoral testing. They include the following: leukopenia among asbestos miners (616); higher lymphocytic adenosine deaminase activity among workers exposed to asbestos (617); induction of aryl hydrocarbon hydroxylase activity in alveolar macrophages and blood lymphocytes in asbestos workers (618); no effect (619) or increased (620) in chromosome alterations in human lymphocytes; and increased in metabolism of polymorphonuclear leukocytes (621).

Asbestos/Benzopyrene Interaction. There are two studies that show conflicting results. Both were designed to obtain proof for the synergism between asbestos and cigarette smoking by examining aryl hydrocarbon hydroxylase activity of pulmonary macrophages and blood lymphocytes obtained from asbestos exposed workers. The first group of study was conducted by a Scandinavian group and showed negative effects: no difference in enzyme induction between smoking and nonsmoking asbestos workers (622, 623). Subsequently, a Texas group of investigators supported by the Council for Tobacco Research, oblivious to the 1980 Swedish publications, found positive proof for the synergism hypothesis (618). This compiler had always

regarded the Swedish work as definitive in nature since the negative results were derived from human cells and would therefore negate the positive results of animal studies [Category 160]. The situation has been changed by the positive results reported by the Texas group. Incidentally, the Texan group is not the Tyler Asbestos Workers Project!

[290] Asbestos/Cigarette Smoking Interactions Derived from Human Postmortem and Antemortem Studies.

The above review of the literature on human pathology and in vitro tests highlights three techniques that support the synergism concept between asbestos and cigarette smoking, namely: [Category 270] foreskin fibroblast culture; [Category 283] rosette formation by lymphocytes; and [Category 285] aryl hydrocarbon hydroxylase of lymphocytes. However additional studies are needed to resolve the question of applicability of conflicting results to epidemiologic studies.

On the other hand, two techniques do not support the asbestos/smoking interaction concept: [Category 241] sputum cytology; and [Category 235] pulmonary content of asbestos bodies and fibers. There is a striking absence of human histopathologic observations applied to the interaction hypothesis, yet the technique has been used to demonstrate the

separate effects of asbestos particles and cigarette smoking on the human lungs.

In a 1982 review of the literature, Craighead and Mossman summarized the biologic mechanisms that would account for the "synergistic carcinogenic effects of asbestos and cigarette smoke in the respiratory tract". Their explanations were derived from the results of their own animal studies [Categories 184, 190 and 192]. The observations by other investigators relating to human pathology and cytology were completely overlooked.

BIBLIOGRAPHY AND CUMULATIVE AUTHOR INDEX

Among the 252 articles for Categories 200s, a duplicate copy of 107 or 42 percent was received from (X). The cumulative total for Categories 100s and 200s is 277 out of 624 articles or 44 percent. The geographical distribution of the authors contained in the Bibliographies are as follows:

United States	Categories 200s		Cumulative
UAR	Arizona		1
UCA	California	11	16
UCT	Connecticut	1	2
UDC	District of Columbia	2	5
UDE	Delaware		2
UIL	Illinois	8	10
UIO	Iowa		3
ULA	Louisiana	3	4
UMA	Massachusetts	2	5
UMD	Maryland	4	19
UME	Maine		1
UMI	Michigan	2	7
UMN	Minnesota	3	14
UMO	Missouri	1	2
UNC	North Carolina	1	10
UNE	Nebraska		2
UNH	New Hampshire	1	1
UNJ	New Jersey	4	10
UNY	New York	17	36
UOH	Ohio	9	17
UPA	Pennsylvania	6	10
USC	South Carolina	2	8
UTE	Tennessee	1	2
UTX	Texas	17	19
UUT	Utah	1	3

UVT	Vermont	2	9
UWA	Washington		6
UWI	Wisconsin		1
UWV	West Virginia	1	3
Total Articles		99	228
Percent of Total		39%	36%

Foreign	Categories 200s		Cumulative
ATL	Australia	4	5
BEL	Belgium	1	2
CAN	Canada	8	19
DEN	Denmark	1	1
EGY	Egypt	4	4
FIN	Finland	4	5
FRA	France	19	38
GBR	Great Britain	62	182
GER	Germany	4	22
IND	India	3	26
ISR	Israel	1	1
ITA	Italy	10	18
JAP	Japan		5
NET	Netherlands	3	4
NOR	Norway	4	4
POL	POland	6	8
ROM	Romania	2	2
RUS	Russia		8
SAF	South Africa	9	33
SWE	Sweden	3	3
SWI	Switzerland	2	2
TAI	Taiwan		1
YUG	Yugoslavia	3	3
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Percent of Total		61%	64%

[Note: Bibliography for categories 200s start on next page]

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In 1967, Kleinfeld et al (660) reported the mortality pattern for 152 asbestos workers in New York State, who had 15 or more years of asbestos exposure by 1945 or had achieved 15 years of exposure to asbestos dust between 1945 and 1965. The observed mortality rates compared to those reported by Selikoff et al were as follows:

CAUSE OF DEATH	NEW YORK STATE NUMBER	%	NEW YORK-NEW JERSEY NUMBER	%
All deaths	46	100.0%	255	100%
All malignancies		50.0%		37%
Lung & pleural malignancies		26.1%		18%
Gastrointestinal & peritoneum		15.2%		11%
All other malignancies		8.7%		...
Cardiac deaths (other than cor pulmonale)		28.3%		...
Asbestosis & complications		4.3%		4%
Residual causes		13.1%		...

In the above mentioned pulication of Kleinfeld et al (660) as well as in subsequent publications (661, 662) appearing respectively in 1968 and 1973, the authors did not mention the asbestos/smoking interaction. Other articles by Kleinfeld relating to asbestos mining are discussed below [Category 339].

[333] Plumbers and Pipefitters Reported by NIOSH Epidemiologists

In 1976, the United Association of Journeymen and Apprentices of the Plumbing and Pipefitting Industries

requested NIOSH to conduct a mortality study of its members. The Union made its computerized death benefit record of 30468 death benefits claimed from 1968 to 1975 available to the NIOSH epidemiologist (663). This represented 10 percent of 330000 members of the union and is 17.8 times larger than the union membership used by Selikoff et al. The proportional mortality ratios (PMRs) for male plumbers and pipefitters combined, and for plumbers only, were as follows:

CAUSE OF DEATH	PMRs PLUMBERS & PIPEFITTERS	PMRs PLUMBERS
All causes	1.00	1.00
All malignancies	1.13	1.27
Digestive organs & peritoneum	1.07	1.25
Esophageal cancer	1.55	2.75
Lung, bronchus & trachea cancer	1.22	1.29

While significant excesses were found for the study group as a whole, these excesses appear to be confined to those union members who were plumbers by trade. Since plumbers, pipefitters and insulators are likely to work together, a question whether common chemical hazards exist needs to be raised. It is also possible that asbestos used by insulators influence plumbers, and vice versa for soldering fumes.

[334] Boilermakers Reported by University of Washington Epidemiologists

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